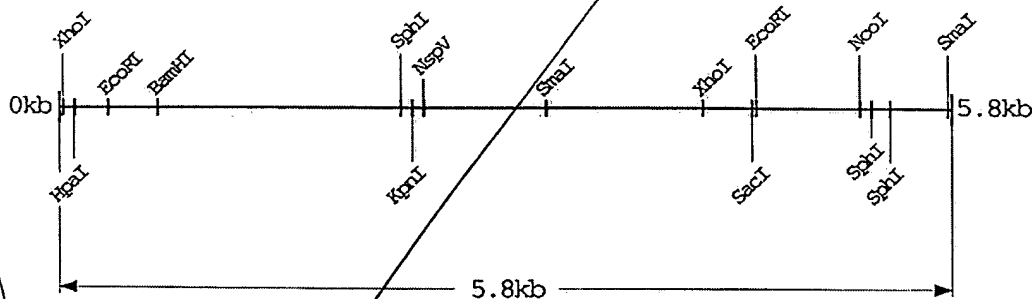


1. (Twice Amended) An isolated DNA fragment of about 5.8 Kb containing a toluene monooxygenase gene, having 1 BamHI restriction site, 2 EcoRI restriction sites, 1 HpaI restriction site, 1 KpnI restriction site, 1 NcoI restriction site, 1 NspV restriction site, 1 SacI restriction site, 2 SmaI restriction sites, 3 SphI restriction sites, 2 XhoI restriction sites, no ClaI restriction site, no DraI restriction site, no EcoRV restriction site, no HindIII restriction site, no NdeI restriction site, no NheI restriction site, no PvuII restriction site, no ScaI restriction site, no Sse8387I restriction site, no StuI restriction site, and no XbaI restriction site, and having a restriction map of:



, said isolated DNA-fragment derived from Burkholderia cepacia KK01.

3. (Three Times Amended) An isolated DNA fragment having a nucleotide sequence that hybridizes under stringent conditions to a hybridization probe with a nucleotide

sequence consisting of SEQ ID NO: 1 or a complement of SEQ ID NO: 1, said DNA fragment
F2 encoding a protein having a toluene monooxygenase activity.

5. (Twice Amended) The recombinant DNA according to Claim 4, wherein the
vector can be maintained or replicated in a bacterium.

6. (Twice Amended) An isolated DNA fragment containing a region encoding a
toluene monooxygenase, the region comprising a first sequence encoding a polypeptide TomL
F3 having an amino acid sequence of SEQ ID NO: 3, a second sequence encoding a polypeptide TomM
having an amino acid sequence of SEQ ID NO: 4, a third sequence encoding a polypeptide TomN
having an amino acid sequence of SEQ ID NO: 5, a fourth sequence encoding a polypeptide TomO
having an amino acid sequence of SEQ ID NO: 6, and a fifth sequence encoding a polypeptide
TomP having an amino acid sequence of SEQ ID NO: 7 of the Sequence Listing, and the first to
fifth sequences are aligned so that expressed TomL - TomP polypeptides can form said toluene
monooxygenase protein.

8. (Twice Amended) An isolated DNA fragment according to claim 6 or 7,
further comprising a sequence encoding a polypeptide TomQ having an amino acid sequence of
F4 SEQ ID NO: 8.

9. (Four Times Amended) An isolated DNA fragment containing a region
encoding a toluene monooxygenase, wherein the region comprises a first sequence that hybridizes

under stringent conditions to a hybridization probe of which nucleotide sequence consists of 463..1455 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomL having an amino acid sequence of SEQ ID NO:3, a second sequence that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 1495..1761 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomM having an amino acid sequence of SEQ ID NO: 4, a third sequence that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 1803..3350 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomN having an amino acid of SEQ ID NO: 5, a fourth sequence that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 3428..3781 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomO having an amino acid sequence of SEQ ID NO: 6, and a fifth sequence that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 3810..4871 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomP having an amino acid sequence of SEQ ID NO: 7, and the first to fifth sequences are aligned so that expressed polypeptides can form said toluene monooxygenase protein.

10. (Four Times amended) An isolated DNA fragment comprising a region that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 234...443 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide having a property of enhanced toluene monooxygenase activity.

13. (Amended) A recombinant DNA comprising an expression vector comprising a first promoter and a first DNA fragment comprising a region that hybridizes under

F5
stringent conditions to a hybridization probe of which nucleotide sequence consists of 234...443 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide having a property of enhanced toluene monooxygenase activity, a second promoter and the DNA fragment according to any one of Claims 6, 7, and 9, wherein the first DNA fragment is functionally linked to the first promoter, and the second DNA fragment is functionally linked to the second promoter.

F6
17. (Three Times Amended) A transformant obtained by introducing a recombinant DNA into a host microorganism, where the recombinant DNA comprises a vector enabling maintenance or replication in a host, said vector including a DNA fragment ligated thereto having a sequence that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of SEQ ID NO: 1 or a complement of SEQ ID NO: 1 and encoding an active toluene monooxygenase, wherein the DNA fragment is 4.9 kb or less encoding a toluene monooxygenase.

F7
19. (Amended) A transformant obtained by introducing a recombinant DNA comprising a vector, a promoter and a DNA fragment into a host microorganism where the DNA fragment contains a region encoding a toluene monooxygenase, the region comprising a first sequence encoding a polypeptide TomL having an amino acid sequence of SEQ ID NO: 3, a second sequence encoding a polypeptide TomM having an amino acid sequence of SEQ ID NO: 4, a third sequence encoding a polypeptide TomN having an amino acid sequence of SEQ ID NO: 5, a fourth sequence encoding a polypeptide TomO having an amino acid sequence of SEQ ID NO: 6, and a fifth sequence encoding a polypeptide TomP having an amino acid sequence of SEQ ID NO: 7, and the first to fifth sequences are aligned so that expressed TomL - TomP polypeptides can form said toluene monooxygenase protein;

F⁷ wherein the promoter and the DNA fragment are functionally linked enabling expression of the toluene monooxygenase protein encoded by the DNA fragment.

F⁸ 21. (Amended) A method for producing a toluene monooxygenase, comprising the steps of:

culturing a transformant according to any one of claims 15, 17 and 19 in a medium; and collecting the expressed toluene monooxygenase.

F⁹ 27. (Amended) The degradation method according to claim 22, wherein the aromatic compound is selected from the group consisting of toluene, benzene, phenol, and cresol.

33. (Amended) The cleaning method according to claim 28 wherein, the aromatic compound is selected from the group consisting of toluene, benzene, phenol, and cresol.

F¹⁰ 34. (Amended) A method for remedying an environment polluted with a pollutant being a chlorinated aliphatic hydrocarbon compound or an aromatic compound, comprising a step of degrading the pollutant by using the transformant according to any one of claims 15, 17 and 19.

F¹¹ 48. (Amended) The remediation method according to claim 34, wherein the aromatic compound is selected from the group consisting of toluene, benzene, phenol, and cresol.